

Genomic DNA extracted from snips of the end of the tails (snips of typically 2 to 5 mm in length) of the fully weaned pups are used to obtain the genotypes of each animal including putative "human-NOS2" transgenic mice (Xu et al. 1996a). After about 3 weeks when the pups are weaned, tail snips are taken and identification tags placed on each animal's ears. Genomic DNA is then extracted from the tails of these F₀ mice (putative founders). Tail snips are minced with a fresh razor blade, placed in 0.4 ml of TE (10 mM Tris-HCl, pH 7.4 and 1 mM EDTA, pH 7.4), extracted twice with 2 volumes of Pheno:chloroform:isoamyl alcohol (50:48:2), re-extracted with 2 volumes of chloroform:isoamyl alcohol (24:1) and re-extracted with 2 volumes of ether. Residual ether in the aqueous phase is removed by drying under a stream of nitrogen gas. The DNA is genotyped by PCR for the presence of the human NOS2 gene using human-specific DNA probes from human NOS2's Promoter/Exon-1 region (Forward Promoter Primer = CCTTTCCTTCCAAAAACCTC, **SEQ ID NO: 1**; Reverse Exon-1 Primer = TCACCCAACCCACCTCTTTC, **SEQ ID NO: 2** to give a 345 bp product). A second PCR from the mouse TAU gene is also performed as a control for the PCR technique and for the presence of mouse DNA using a forward primer (TAU Exon-14 Forward Primer = TTGGCACTTCGATGATGACCTC, **SEQ ID NO: 3**) and reverse primer (TAU Exon-14 Reverse Primer = CATTGTGACGTGTGATGAGGG, **SEQ ID NO: 4**) which give a PCR product of 420 bp whose sequence matches that reported (Andreadis et al., 1992). Southern blots of genomic DNA digested with Hind III and hybridized with the pIN-2 probe (spanning positions +2401 to +4203 of the human NOS2 cDNA which corresponds to Exon-18 to Exon-27 of the human NOS2 gene), are also employed. In the Southern paradigm, human NOS2 gene gives multiple bands of 12, 9.5, 8, 6, 4.5, 4, and 3.6 kBp, while mouse genomic DNA gives only one band of 7.6 kBp (Xu et al. 1994). Hybrid mice containing the human NOS2 and the mouse NOS2 genes contain the mouse-specific 7.6 kBp band and all 7 of the human-specific bands. In an alternative Southern paradigm, BamHI digest of genomic DNA probed with oligonucleotides from the sequence of Exon-18 are predicted to give a 14 kBp band from wild-type mouse DNA, a 10 kBp band from mu-NOS2-knockout DNA and a 2 kBp band from human DNA (Laubauch et al. 1995; Xu et al. 1996b). Non-transgenics have a 14 kBp band hybridizing to an Exon-18 probe, while the hemizygous "human NOS2" transgenic mouse displays bands at 14 kBp and at 2 kBp hybridizing to the Exon-18 probe.